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Impact of *G. applanatum*, a multifunctional herbal extract on haematological profiles of laboratory rats: a preliminary study in Nigeria

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ABSTRACT

Objective: To ascertain the haematological properties of aqueous extract of *G. applanatum*(*G. applanatum*). **Methods:** Sixty albino rats were grouped into six equal groups (10 each) from A to F, consisting of tests and controls. Laboratory albino rats in groups A, B and C were infected with *Trypanosoma brucei* (*T. brucei*) while groups A and B (Test) were treated with aqueous *G. applanatum* extract; other groups served as control. Microscopy and haematological profiles from the albino rats were monitored on daily basis for blood parasites, Packed Cell Volume (PCV), Haemoglobin Concentration (HC), Total Red Blood Cell Count (RBC), Mean Cell Haemoglobin (MCH), Mean Cell Volume (MCV), Mean Cell Haemoglobin Concentration (MCHC), and Total White Blood Cell Count (WBC). **Results:** Albino rats in groups A, B and C infected with *T. brucei* and treated with various concentrations of aqueous *G. applanatum* showed a progressive reduction in PCV, HC, RBC, MCH and MCHC compared to the controls ($P< 0.05$). All the infected rats died by day 14 of the experiment from parasitaemia. **Conclusions:** *G. applanatum* lacks ability to boost haematological profiles of anaemic laboratory rats and also of no use in the treatment of African Trypanosomiasis. Higher doses of the fungal extract may be required to test on laboratory rats with less lethal biological stimulants of anaemia before proving or otherwise its true haematological properties.

1. Introduction

Ganoderma species belong to the genus Basidiomycete of the higher fungi. It has a global distribution as taxonomists have traced its pan-continental presence for several centuries in the past[1-3]. Its medicinal value is also not new as it can probably be traced to the Greek, Medieval, Persian as well as the rich Chinese herbal medicine dating as far back as 2500 BC[4-6].

Ganoderma has a unique double walled basidiospore with a shining skin. Some of the active compounds identified in the cell wall of the mushrooms include protein-bound polysaccharides and long chain glucose[7-9]. These compounds along with probably others have been found useful in the treatment of malignancies such as leukaemias as well as immunodeficiency states. Similarly extracts of *Ganoderma lucidum* (*G. lucidum*) specifically have been found useful in the treatment of viral, bacterial as well as some parasitic infections and infestations[10-12].

In Nigeria as well as several other parts of Sub-Saharan Africa, the pharmacologic potential of *G. lucidum* appear grossly underutilized even in its crude form as there is little available literature on its activity[13-15]. This study is therefore set up to ascertain the haematological impact of *G. applanatum* on albino-rats induced anaemia[16-18]. The findings would be useful as preliminary information when more attention is eventually drawn to exploit the medicinal benefits inherent in the fungus.

2. Materials and methods

2.1 Sample collection

The study was carried out in Federal College of Veterinary

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and Medical Laboratory Technology, National Veterinary Research Institute, Vom, Nigeria. Experimental rats were obtained from Nigerian Institute for Trypanosomiasis Research (NITR). The rats were kept in laboratory cages, fed with commercially prepared and vital feeds and allowed to acclamatise for four weeks. Blood samples were then collected from the tail vein on a microscope slide and examined under the microscope to exclude the presence of trypanosomes. Also *Trypanosoma brucei* (*T. brucei*) infected laboratory rats were obtained from NITR, Vom.

2.1. G. applanatum extraction

The powder of *G. applanatum* 1 kg was dissolved in 3 L of distilled water. The sample was boiled for 3 h, stirred every thirty minutes. It was then allowed to stand for 24 h and then filtered using whatman number 1 paper. The filtrate was evaporated to dryness in hot air oven set at 45℃, the extract obtained was reconstituted using sterile distilled water to obtain concentrations 500 mg/mL and further diluted to obtain 250 mg/mL[19].

2.2. Source of T. brucei

Albino rat as parasite donor was obtained from NITR, Vom. Blood (0.5 mL) was collected from the parasite donor rat and diluted with normal saline (50:50). A drop of the diluted blood was examined under the microscope to ensure that there was presence of the parasites. The parasitaemia examined was on the average of 5/field. 0.1 ml of the diluted was used for injecting the infected group of albino rats intraperitoneally[20].

2.3. Rat groupings

Sixty rats were grouped into six with 10 rats in each group.

Group A- Rats infected and treated with 250 mg b.w. of aqueous *G. applanatum* extract of the rats.

Group B- Rats infected and treated with 500 mg b.w.of aqueous *G. applanatum* extract of the rats.

Group C-Rats infected and not treated with *G. applanatum* extract.

Group D- Rats uninfected but treated with 250 mg b.w.of aqueous *G. applanatum* extract.

Group E- Rats uninfected but treated with 500 mg b.w. aqueous *G. applanatum* extract.

Group F- Rats uninfected and untreated.

T. brucei was used to induce anaemia in albino rats infected with the parasites. Group C served as positive control while group F served as negative control.

2.4. Blood sample collection

Rats used in the study were bled through the ocular vein into Ethylene diamine tetra-acetic acid bottles. The samples were analysed immediately in Haematology and Microbiology Laboratories of Federal College of Veterinary and Medical Laboratory Technology, Vom.

Estimation of haematocrit packed cell volume (PCV)- blood was collected using capillary tubes (length of 75 mm and diameter of 1 mm) by capillary action, leaving 15 mm unfilled. The tubes were sealed by flaming and spun in a microhaematotocrit centrifuge at 1 200 g for 5 minutes. PCV was then measured using haematocrit reader[21].

Haemoglobin estimation- A 1:250 dilution of blood was made by adding 0.02 mL of blood to 5 mL of Drabkins solution in a test tube. This was mixed and allowed to stand for 5 minutes, for complete conversion. The test was read colorimetrically at 540 nm.

White blood cell count (WBC)- A 1:20 dilution of blood was made by adding 0.02 mL of blood to 0.38 ml of Turks solution in a 75×10 mm plastic tube. After tightly corking the tube the suspension was well mixed by rotation. The improved Neubauer counting chamber was loaded with the diluted blood by means of pasteur pipette. The loaded counting chamber was allowed for two minutes for cells to settle, after which the preparation was viewed under the microscope ×10 mm objective. The cells were counted in the 4 large corner squares of the counting chamber. The calculation of total white blood cells was made using the first principle[22].

Red blood cell (RBC) count- A 1:200 dilution of blood was made in formol citrate solution by diluting 200 mL of blood into 4 mL of diluents in a plastic tube. A clean dry improved Neubauer counting chamber with cover slip already in position was loaded with diluted blood using Pasteur pipette. The chamber was left undisturbed for 2 minutes for the cells to settle. The cells were counted under the microscope using X10 mm objective. Cells were counted in 80 small squares in the central ruled area of the counting chamber[22].

2.5. Data Analysis

Data obtained was analysed using simple descriptive methods of Arithmetic mean, mode and Standard deviation (SD) as well as Epi Info statistical software 2006 version.

3. Results

Albino rats in groups A, B and C infected with *T. brucei* and treated with various concentrations of aqueous *G. applanatum* showed a progressive reduction in PCV, haemoglobin concentrations (HC), RBC, mean cell haemoglobin (MCH) and mean cell haemoglobin concentrations (MCHC) compared to the controls ($P< 0.05$).

There was no significant change in mean cell volume (MCV) and total white blood counts among the treated

Table 1
PCV, HC, TRC of rats infected with *T. brucei* and treated with aqueous *G. applan*atum extract.

Groups	PCV(%)			HC (g/dl)			TRC ($\times 10^{12}$)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Group A	47.4± 0.28	23.4± 0.18	Died	13.3±0.57	7.3±0.61	Died	5.91±1.65	2.10±3.39	Died
Group B	46.0± 0.22	21.7± 0.23	Died	13.6±0.10	5.8±0.60	Died	6.78±0.55	3.42±3.66	Died
Group C	46.6± 0.35	19.0± 1.6	Died	12.8±0.68	5.1±0.42	Died	6.62±0.51	2.87±2.41	Died
Group D	36.3± 1.36	29.6± 1.92	34.6± 1.04	10.1±0.35	8.5±0.56	11.3±0.42	5.01±1.57	4.23±3.10	4.99±1.18
Group E	31.9± 1.24	16.1± 0.52	46.1± 0.40	9.4±0.27	4.3±0.26	14.1±0.64	4.80±1.37	2.15±1.37	5.99±0.94
Group F	36.0± 0.35	14.8± 0.91	44.0± 0.07	10.6±0.16	4.1±0.28	12.6±0.14	5.21±0.49	1.97±0.25	6.36±0.83

Table 2
TWC count, MCV, MCH of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Groups	TWC (0 x10^9)			MCV			MCH (pg)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Group A	6.93± .24	6.55±2.84	Died	67.1±3.84	66.9±6.3	Died	20.0±1.15	11.4±0.57	Died
Group B	9.70±3.33	8.10±2.40	Died	65.5±1.95	63.7±0.64	Died	19.3±0.64	10.8±2.69	Died
Group C	8.80±1.03	7.40±3.36	Died	69.0±1.57	66.4±4.85	Died	20.1±1.66	9.0±1.31	Died
Group D	6.83±0.45	4.83±2.80	10.33±0.24	69.8±1.30	72.2±8.66	75.9±5.05	19.9±1.20	20.1±2.88	22.1±3.61
Group E	6.83±0.45	6.20±1.05	10.96±2.46	66.3±2.75	77.1±4.96	76.9±3.28	18.8±1.80	20.4±1.01	23.7±2.78
Group F	6.85±0.49	3.55±2.33	6.90±0.35	69.2±0.42	75.0±1.41	71.9±6.57	25.9±2.02	20.7±0.85	20.55±2.93

Table 3
MCHC (g/l) of rats infected with *T. brucei brucei* and treated with aqueous *G. applanatum* extract.

Day	Group A	Group B	Group C	Group D	Group E	Group F
0	299.0±15.58	292.8±13.15	312.5±47.78	286.3±18.01	284.0±33.87	303.5±31.82
7	162.0±15.56	146.5±41.72	173.0±6.24	279.0±19.08	266.3±11.93	278.5±3.54
14	Died	Died	Died	291.0±42.32	308.0±14.53	286.0±1.42

infected rats compared to the controls (P> 0.05).

Albino rats in group A, B and C all died from day 12 to day 14.

There was no significant change in haematological profiles tested among albino rats uninfected with *T. brucei* but treated with *G. applanatum* in group E similar to those in group F who were neither infected nor treated with the *G. applanatum* extract during the study period (P > 0.05), (Tables 1-3).

The total white cell count of rats in groups D and E which were uninfected but treated with *G. applanatum* increased significantly at day 14 when the experiment was terminated (P< 0.05) .

The experiment was terminated at day 14 when all the test rats and those ones in positive control died.

4. Discussion

T. brucei (federe) is a tissue parasite which induces anaemia in infected rats and other susceptible animals such as cattle, dogs and mice[20,23,24]. This manifested in the fall in PCV, MCH, MCHC and total red cell counts among the test animals and the positive controls in the present study(P< 0.05). Although the bleeding intervals of the rats may also have affected the haematological parameters, the non-significance of this effect as seen in the negative controls stresses the negligible effect on the overall result[25,26].

The total WBC showed no significant decrease in rats infected with *T. brucei* and treated with *G. applanatum* (P> 0.05) but significant decrease in infected but untreated rats (P< 0.05). This is in line with immunopotentiation and immunomodulatory properties severally attributed to *Ganoderma species* which have found wide clinical applications in the management of malignancies and immunodeficiency states[27-29].

All the test and control rats infected with *T. brucei* died between day 12 to day 14 primarily due to overwhelming parasitaemia and probably secondary anaemia. This points to the fact that the Ganoderma extracts had no therapeutic effect on *T.brucei* contrary to its established antibacterial, antiviral, antimycotic and other anti-infectious applications[30, 31]. Higher doses may still need to be tried to ascertain the true usefulness or otherwise of this fungus in the management of Trypanosomiasis.

The full impact of aqueous *Ganoderma species* extract on the haematological profiles of rats in the present study which was originally designed to last for a minimum of 28 days was terminated on day 14 when all the test animals died about midway into the test period. The healthy appearance and agility of all uninfected rats equally treated with aqueous *Ganoderma species* at day 14 and beyond implies all the test rats did not die from Ganoderma toxicity[32,33].

It is indeed our candid view that the effect of extract of this fungus on haematological parameters would probably have been more pronounced and conclusive had the

rats survived the infection beyond day 14 up to 28th day. Further studies using less lethal biological agents to induce anaemia in rats is therefore required to fully study the haematological properties of *Ganoderma species*, The fact that the haematological parameters of uninfected rats picked up by day 14 further strengthens this view[34,35].

In conclusion, *G. applanatum* extracts failed to correct anaemia induced by *T. brucei* in rats and also failed to kill the parasites, although all the test animals died midway into the period of experiment. Higher concentrations of aqueous *Ganoderma species* extract may therefore be tried to fully establish the activity of the fungus or otherwise in this regard. Similarly, its level of bioavailability in rats should be assessed to ascertain its suitability as a potential candidate drug for the treatment of haemoparasites such as African Trypanosomiasis as well as its ability to boost blood parameters.

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Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Fang YC, Huang HC, Chen HH, Juan HF. TMC Gene DIT: a database for associated Chinese medicine, gene and disease information using text mining. *BMC Complem Altern Med* 2008; 8: e58. Doi: 10.1186/1472-6882-8-58.

[2] Luo H, Sun C, Song J, Lan J, Li Y, Li X et al. Generation and analysis of expressed sequence tags from a cDNA library of the fruiting body of *Ganoderma lucidum*. *Clin Med* 2010; **5**: e9. doi: 10.1186/1749-8546-5-9.

[3] Cheng KC, Huang HC, Chen JH, Hsu JVV, Cheng HC, Ou CH, et al. *Ganoderma lucidum* polysaccharides in human monocytic leukaemia cells: from gene expression to network constriction. *BMC Genomics* 2007; **8**: e411.

[4] Grinn-Gofron A, Strzelezak A. The effects of meteorological factors in the occurrence of Ganoderma spp. spores in the air. *Int J Biometeorol.* 2011; **55**(2): 235-241.

[5] Yeh CM, Yeh CK, Hsu XY, Luo QM, Lin MY. Extracellular expression of a functional recombinant *Ganoderma lucidum* immunomodulatory protein by Bacillus subtilis and Lactococcus lactis. *Appl Environ Microbiol* 2008; **74**(4): 1039-1049.

[6] Stajich JE, Berbee ML, Blackwell M, Hibbett DS, James TY, Spatafora JW, Taylor JW. *Primer- The Fungi. Curr Biol* 2009; **19**(18): R840-R845.

[7] Fernandez RG, Prats E, Novo JVJ. Proteomics of plant pathogenic fungi. *J Biomed Biotechnol.* 2010; 2010: e932527. doi: 10. 1155/2010/932527.

[8] Ajith TA, Janardhanan KK. Indian medicinal mushrooms as a source of antioxidant and antitumour agents. *J Clin Biochem Nutr.* 2007; **40**(3): 157-162.

[9] Ko CM, Leung HY. Enhancement of ATP generation capacity, antioxidant activity and immunomodulatory activities by Chinese Yang and Yin tonifying herbs. *Clin Med.* 2007; 2: e3. doi: 10. 1186/1749-8546-2-3.

[10] Gbolagade SJ, Awotona FE. Studies on antimicrobial potentials of three *Ganoderma species* collected from University of Ibadan (Nigeria) botanical gardens. Afri J Biomed Res. 2010; 13(2). http://www.ajbrui.net/index.php/ajbr/issue/view/2.

[11] Breemen RB, Fong HHS, Farnsworth NR. The role of quality assurance and standardization in the safety of botanical dietary supplements. *Chem Res Toxicol.* 2007; **20**(4): 577-582.

[12] Rodriguez ML, Nimrichter L, Cordero RJB, Casadevall A. Fungal polysaccharides, biological activity beyond the usual structural properties. Front Microbiol. 2011; 2: e171. doi: 10. 3389/fmicb.2011.00171.

[13] Jombo GTA, Enenebeaku MNO, Ayeni JA, Fagbemi OI. Invitro susceptibilities of clinical isolates of yeast and yeast-like fungi to various extracts of Jatropha curcas. *Continental J of Microbiology.* 2007; **1**: 13-21.

[14] Anthony MM, Joyce C. Proximate and minimal composition of four edible mushrooms from South Western Nigeria. *Afric J Biotechnol.* 2007; **4**(10): 1084-1088.

[15] Jombo GTA, Enenebeaku MNO. Antimicrobial profile of fermented seed extracts of Ricinus communis: Findings from a preliminary analysis. *Niger J of Physiological Sciences.* 2008; **23**(1&2): 55-59.

[16] Ko KM, Leung HY. Enhancement of ATP generation capacity, antioxidant activity and immunomodulatory activities by Chinese Yang and Yin tonifying herbs. *Chin Med.* 2007; **2**: e3.

[17] Schwan WR, Dunek C, Gebhardt M, Engelbrecht K, Klett T, Monte A, Toce J, Rott M, Volk TJ, LiPuma JJ, Liu XT, McKelvey R. Screening a mushroom extract library for activity against Acinetobacter baumannii and Burkholderia cepacia and the identification of a compound with anti-Burkholderia activity Ann Clin Microbiol Antimicrob. 2010; 9: e4. doi: 10.1186/1476-0711-9-4.

[18] Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Appl Microbial Biotechnol,* 2011; **89**(5): 1323-1332.

[19] Naso FC, Mello RN, Bona S, Dias AS, Porawski M, Ferraz ABF, Richter MF, Marroni NP. Effect of Agaricus blazei Murill on the Pulmonary Tissue of Animals with Streptozotocin-Induced Diabetes. *Exp Diabetes Res.* 2010; 2010: e543926. doi: 10.1155/2010/543926.

[20] Wissmann B, Machila N, Picozzi K, Fèvre EM, Bronsvoort BMC, Handel IG, Welburn SC. Factors Associated with Acquisition of Human Infective and Animal Infective Trypanosome Infections in Domestic Livestock in Western Kenya. PLoS Negl Trop Dis. 2011; 5(1): e941. doi: 10.1371/journal.pntd.0000941.

[21] Luckins AG. Methods for diagnosis of trypanosomiasis in Livestock. http://www.fao.org/warcent/facinfo/agricult/aga/AGAP/FRG/Feedback/war/u6680b/u66oboa. Accessed 9th June

2011

[22] MacLean LM, Odiit M, Chisi JE, Kennedy PGE, Sternberg JM. Focus-Specific Clinical Profiles in Human African Trypanosomiasis Caused by Trypanosoma brucei rhodesiense. *PLoS Negl Trop Dis*. 2010; **4**(12): e906. doi: 10.1371/journal.pntd.0000906.

[23] Blom-Potar MC, Chamond N, Cosson A, Jouvion G, Droin-Bergère SD, Huerre M, Minoprio P. Trypanosoma vivax Infections: Pushing Ahead with Mouse Models for the Study of Nagana. II. Immunobiological Dysfunctions. PLoS Negl Trop Dis. 2010; 4(8): e793. doi: 10.1371/journal.pntd.0000793

[24] Chamond N, Cosson A, Blom-Potar MC, Jouvion G, D'Archivio S, Medina M, Droin-Bergère S, Huerre M, Goyard S, Minoprio P. Trypanosoma vivax Infections: Pushing Ahead with Mouse Models for the Study of Nagana. I. Parasitological, Hematological and Pathological Parameters. PLoS Negl Trop Dis. 2010; 4(8): e792. doi: 10.1371/journal.pntd.0000792

[25] Roy N, Nageshan RK, Pallavi R, Chakravarthy H, Chandran S, Kumar R et al. Proteomics of Trypanosoma evansi Infection in Rodents. *PLoS One* 2010; **5**(3): e9796. doi: 10.1371/journal.pone.0009796.

[26] Noyes HA, Alimohammadian MH, Agaba M, Brass A, Fuchs H, Gailus-Durner V et al. Mechanisms Controlling Anaemia in *Trypanosoma congolense* Infected Mice. *PLoS ONE,* 2009; **4**(4): e5170. doi: 10.1371/journal.pone.0005170

[27] Ding Y, Seow SV, Huang CH, Liew LM, Lim YC, Kuo IC, et al. Coadministration of the fungal immunomodulatory protein FIP-Fve and a tumour-associated antigen enhanced antitumourimmunity Immunology. 2009; **128**(1pt2): e881-e894.

[28] Razumov IA, Kosogova TA, Kazachinskaia EI, Puchkova LI, Shcherbakova NS, Gorbunova IA et al. Antiviral activity of aqueous extracts and polysaccharide fractions from mycelium and fruit bodies of higher fungi. *Antibiot Khimioter* 2010; **55**(9-10):14-18.

[29] Jiang J, Slivova V, Sliva D. *Ganoderma lucidum* inhibits proliferation of human breast cancer cells by down-regulation of oestrogen receptor and NF-kappat signalling. *Int J Oncol* 2006; **24**: 695-703.

[30] Adams M, Christen M, Plitzko I, Zimmermann S, Brun R, Kaiser M, Hamburger M. Antiplasmodial lanostanes from the *Ganoderma lucidum* mushroom. *J Nat Prod* 2010;**73**(5):897-900.

[31] Sudisha J, Shetty HS. Anti-oomycete compounds from Ganoderma appalantum, a wood rot *basidiomycete*. *Nat Prod Res* 2009;**23**(8):737-753.

[32] Liu YW, Gao JL, Guan J, Qian ZM, Feng K, Li SP. Evaluation of antiproliferative activities and action mechanisms of extracts from two species of Ganoderma on tumor cell lines. *J Agric Food Chem* 2009; **57**(8):3087-3093.

[33] Noguchi M, Kakuma T, Tomiyasu K, Yamada A, Itoh K, Konishi F, Kumamoto S, Shimizu K, Kondo R, Matsuoka K. Randomized clinical trial of an ethanol extract of *Ganoderma lucidum* in men with lower urinary tract symptoms. *Asian J Androl.* 2008 Sep;**10**(5):777-785.

[34] Fèvre EM, Wissmann Bv, Welburn SC, Lutumba P. The Burden of Human African Trypanosomiasis. *PLoS Negl Trop Dis* 2008; **2**: e333.

[35] Fèvre E, Odiit M, Coleman P, Woolhouse M, Welburn S. Estimating the burden of rhodesiense sleeping sickness during an outbreak in Serere, eastern Uganda. *BMC Pub Health* 2008; **8**: e96.